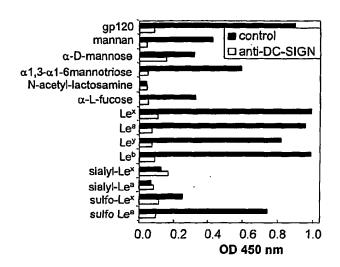
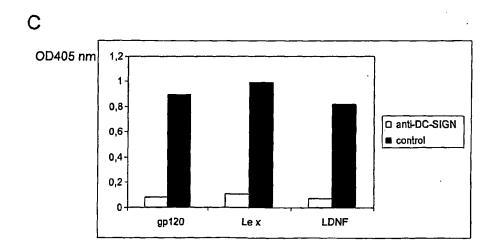
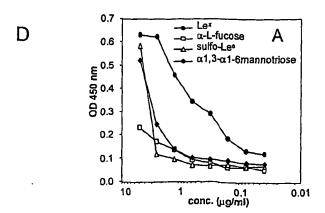
Fig. 1

#### A Is table of structures

В

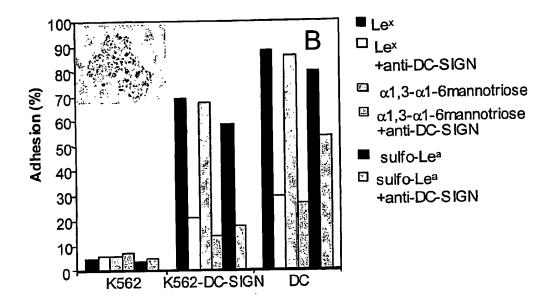






BEST AVAILABLE COPY

Fig. 2



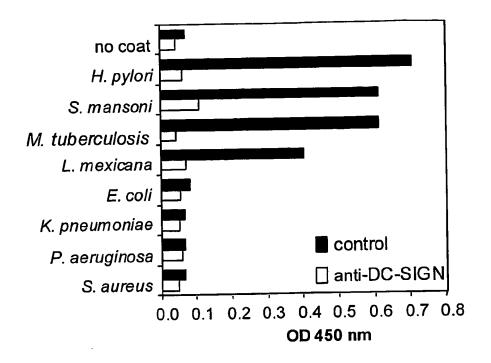


Fig. 3A

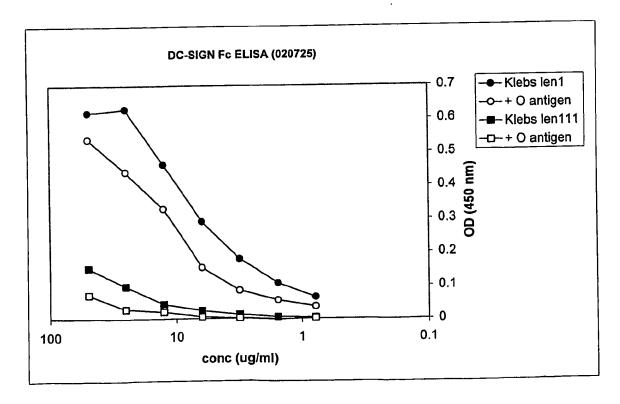


Fig. 3B

Fig. 4

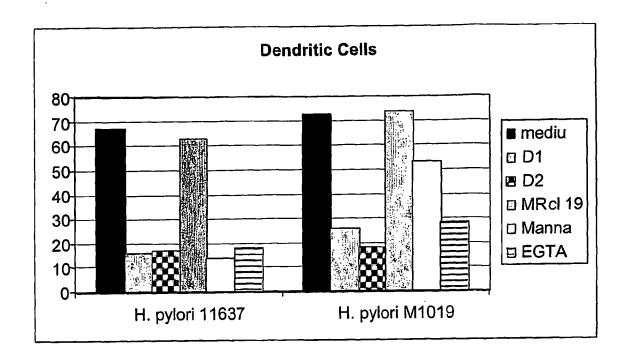


Fig. 5

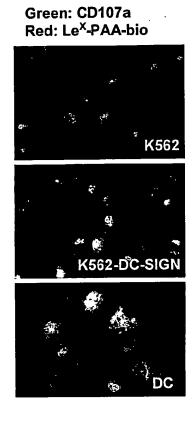


Fig. 6

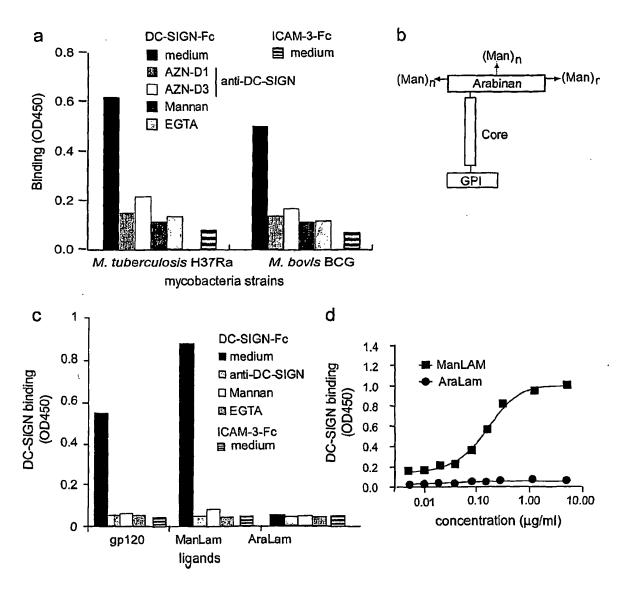
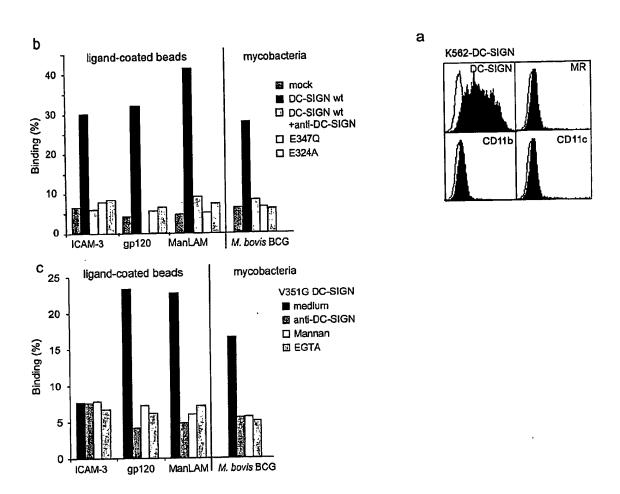


Fig. 7



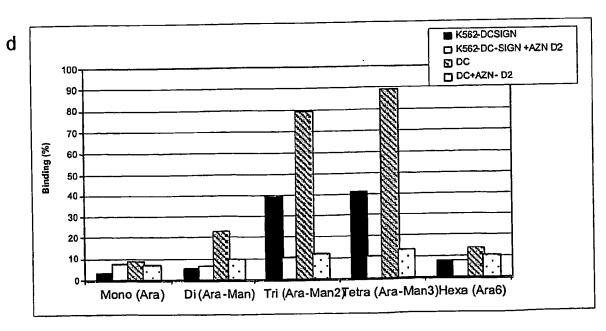


Fig. 8

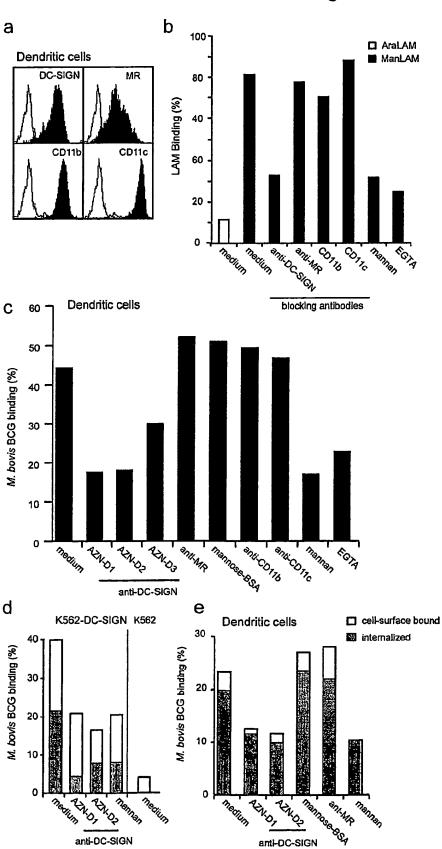
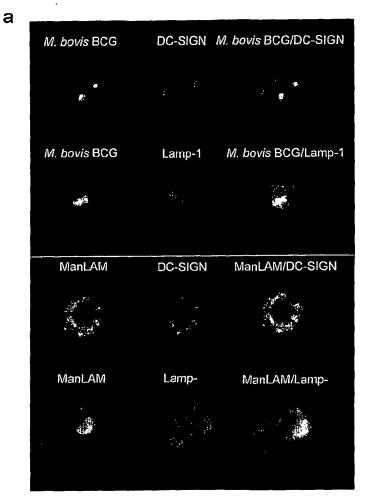


Fig. 9



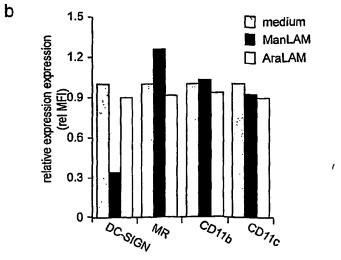
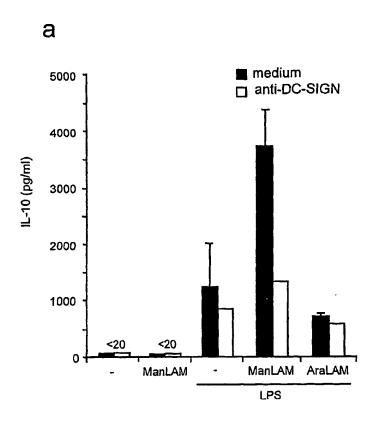


Fig. 10



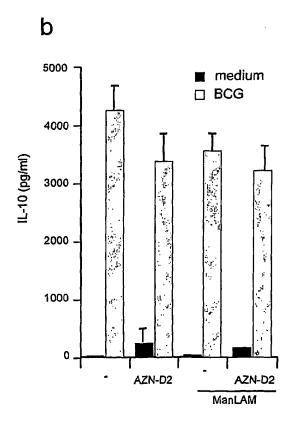


Fig. 11

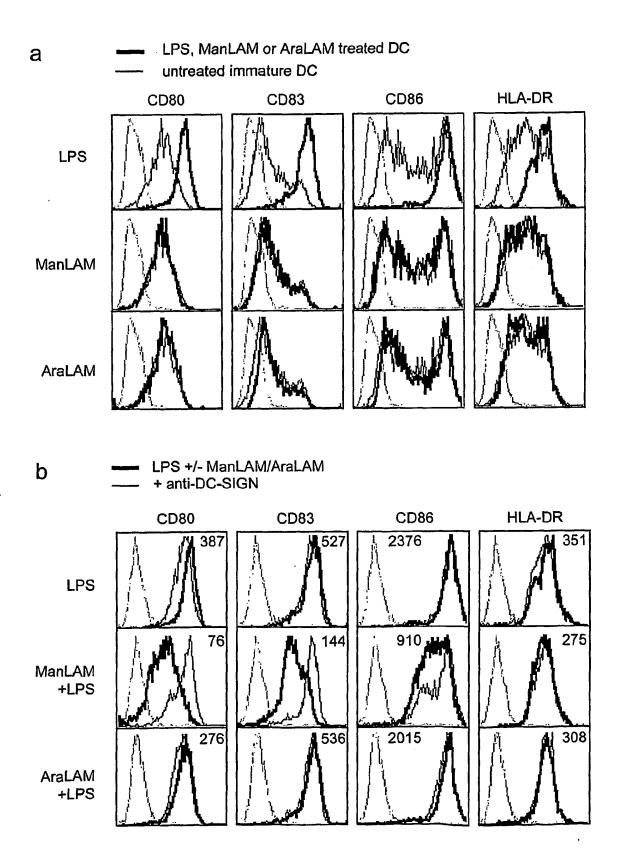
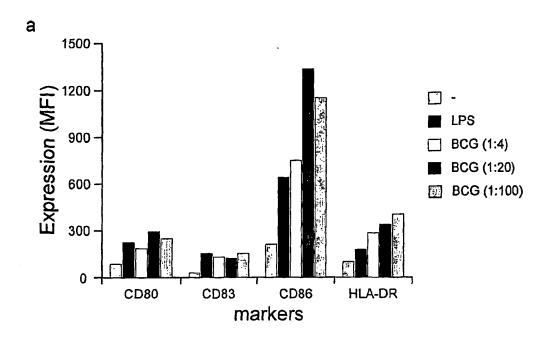


Fig. 12



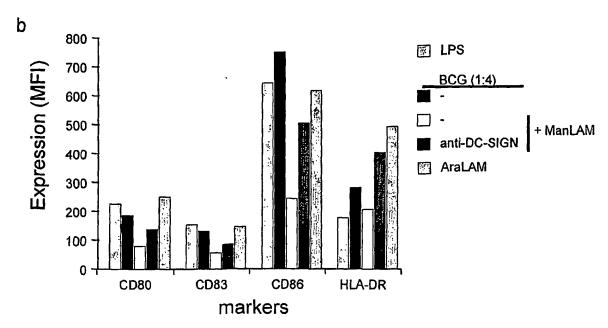


Fig. 13

Carbohydrate antigens on S. mansoni SEA

Carbohydrate epitope	Shortname	anti-glycan MAb		
	Lewis-X	CB10 (32)		
<u>β4</u> ■ R	LDN	SMLDN1.1 (31)		
$\Box_{\overline{\beta4}}^{\bigcirc \alpha3} = R$ $\Diamond_{\alpha2}$	LDNF	SMLDNF1 (4)		
α3 	LDN-DF	114-5B1-A (12)		
● Gal ■ GlcNAc ☐ GalNAc △ Fucose				

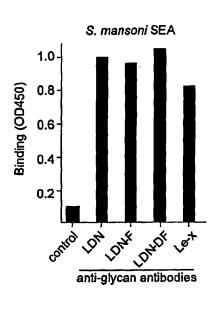
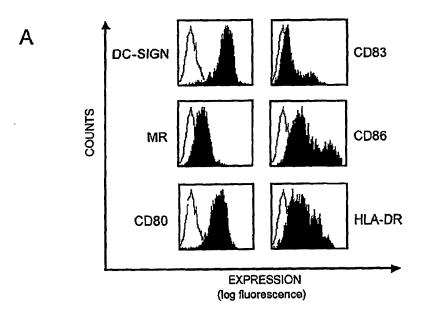


Fig. 14



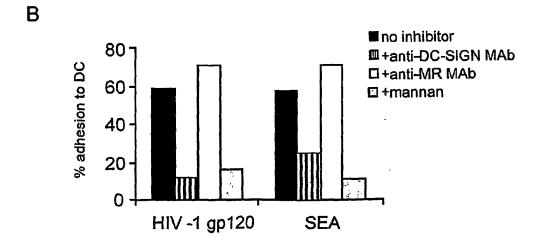


Fig. 15

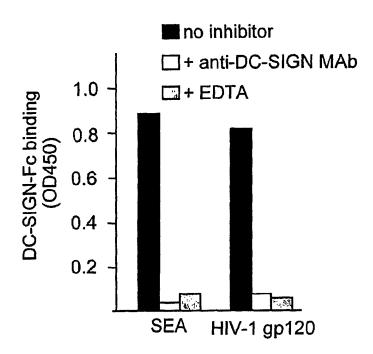


Fig. 16

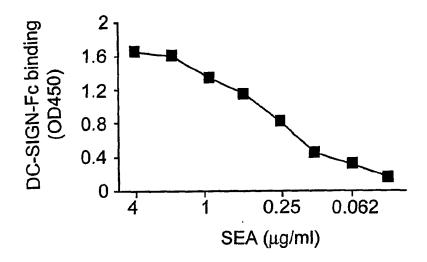
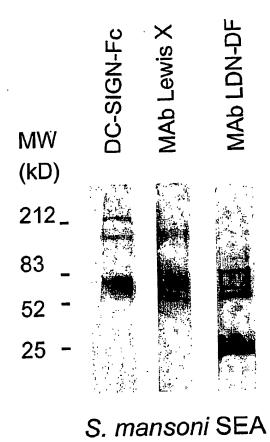
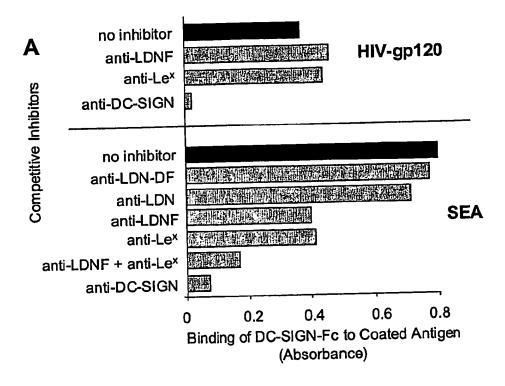


Fig. 17



SUBSTITUTE SHEET (RUI F 26)

Fig. 18



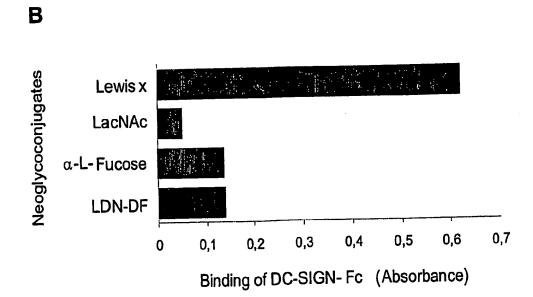
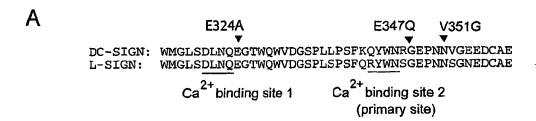
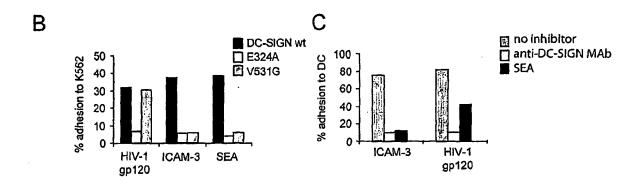
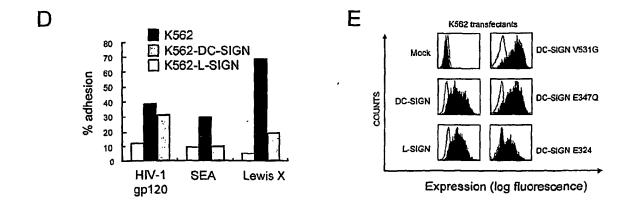


Fig. 19







PCT/NL2003/000781 10/533981

Fig. 20A

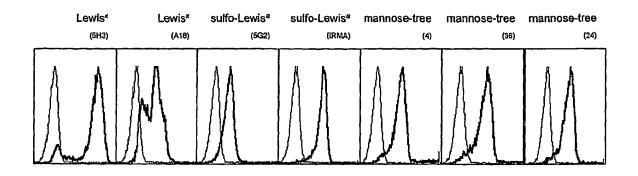


Fig. 20B

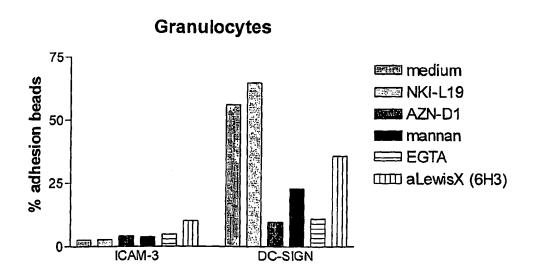
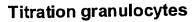
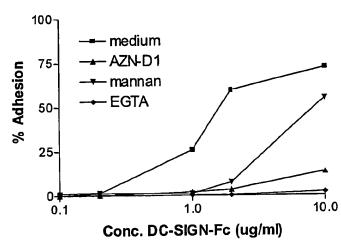


Fig. 21





### **Granulocytes**

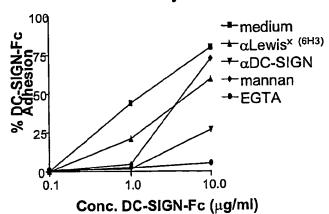


Fig. 22A

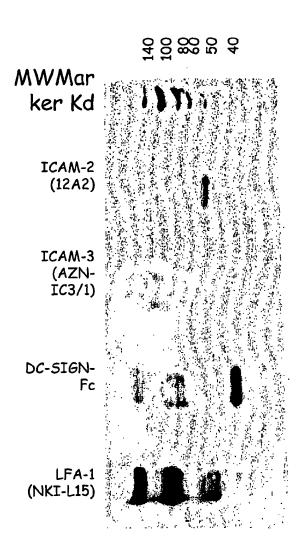


Fig. 22B

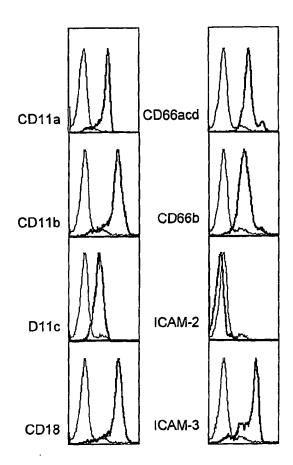
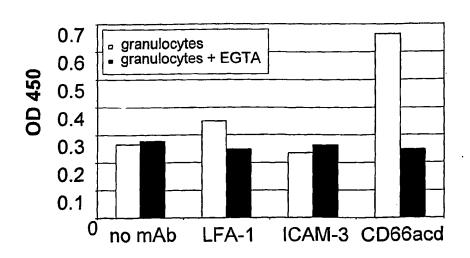
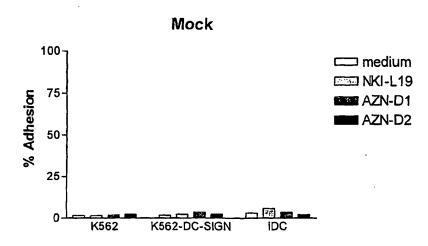


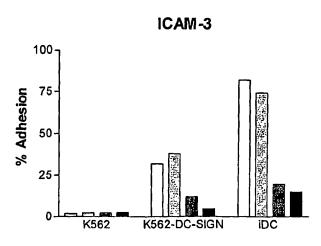
Fig. 22C



SUBSTITUTE SHFFT (RIII F 26)

Fig. 23





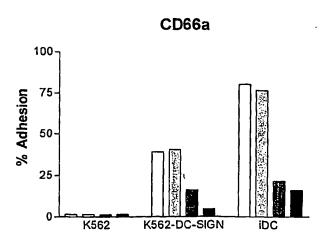


Fig. 24

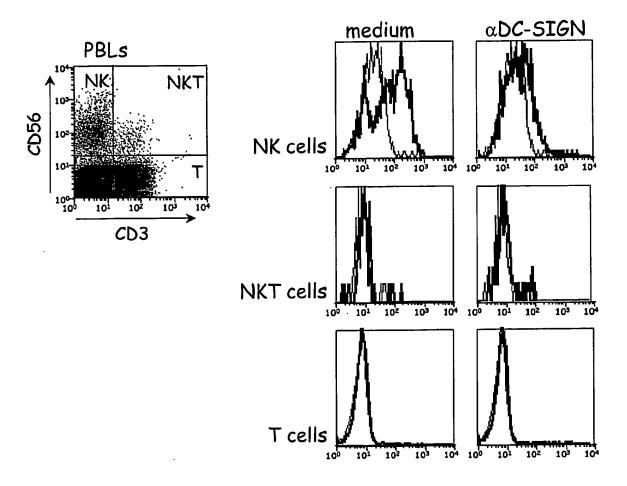


Fig. 25

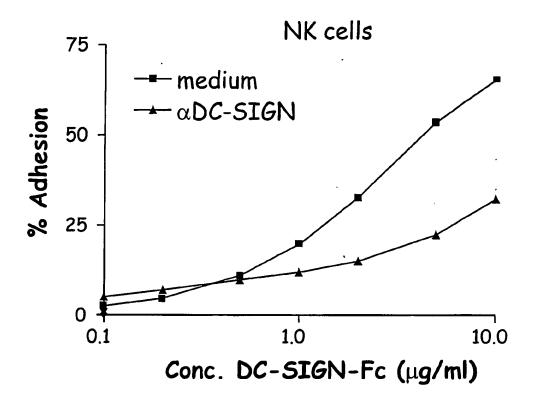


Fig. 26

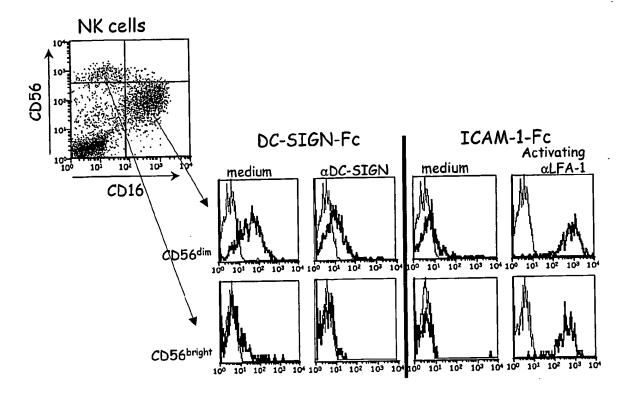
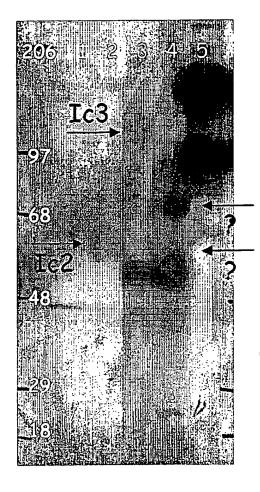


Fig. 27



- 1. control
- 2. ICAM-2
- 3. ICAM-3
- 4. DC-SIGN ligand
- 5. LFA-1

→ DC-SIGN binds
ICAM-2? and 75
kD ligand on NK
cells

Fig. 28

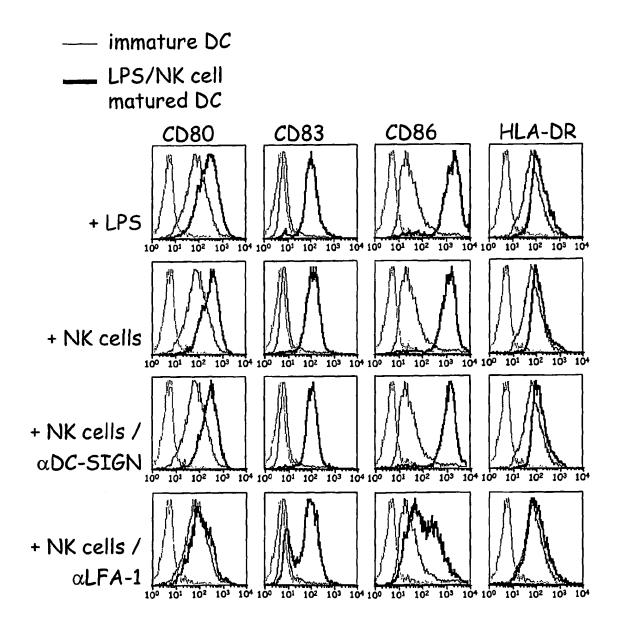


Fig. 29

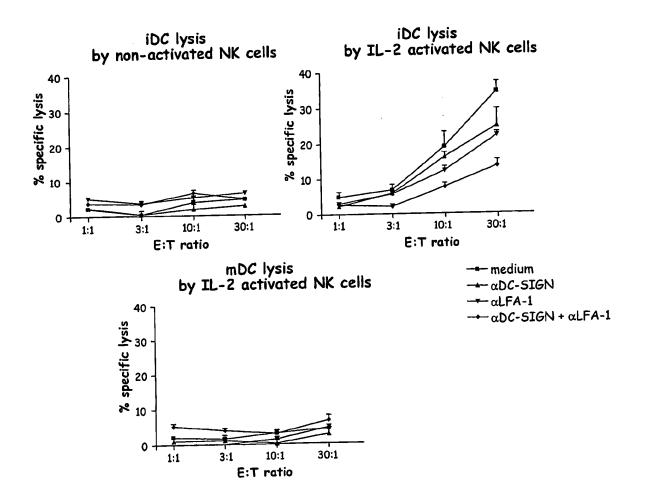


Fig. 30

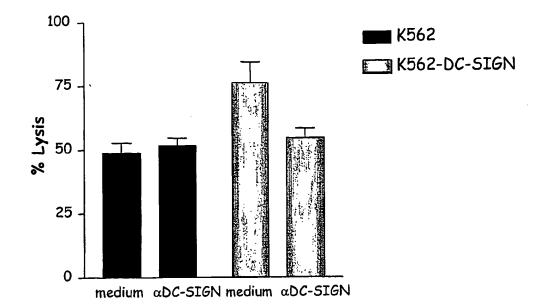
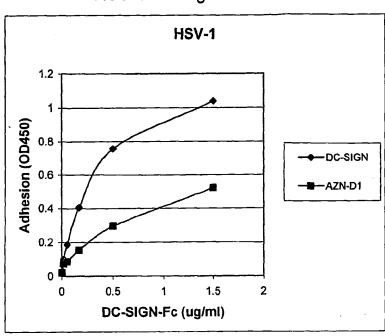


Fig. 31

**HSV-1** binding



HSV-2 binding

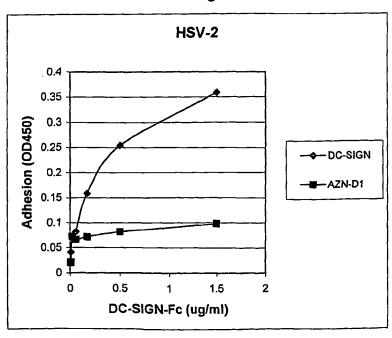
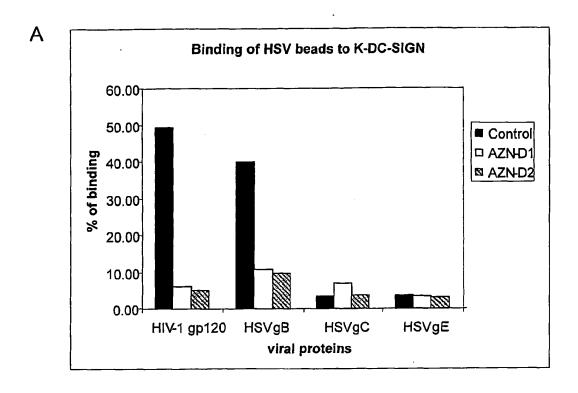


Fig. 32



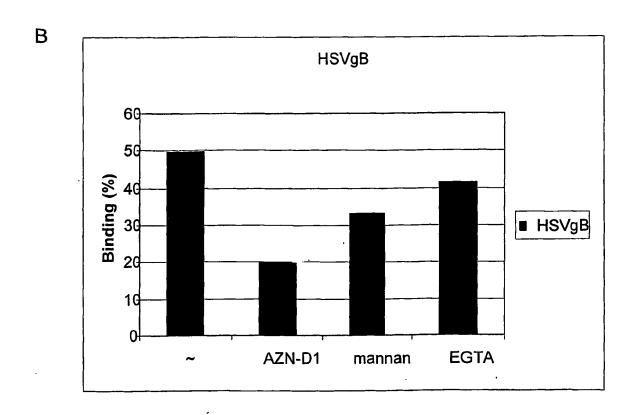


Fig. 33A

# mSIGNR1 binds mannose-containing carbohydrates, similar to DC-SIGN and L-SIGN

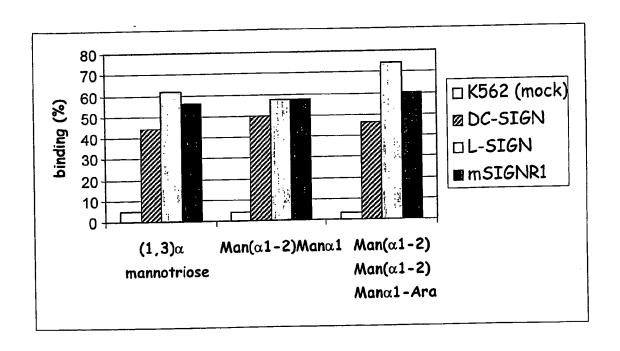


Fig. 33B

DC-SIGN, L-SIGN and mSIGNR1 bind differently to Lewis antigens

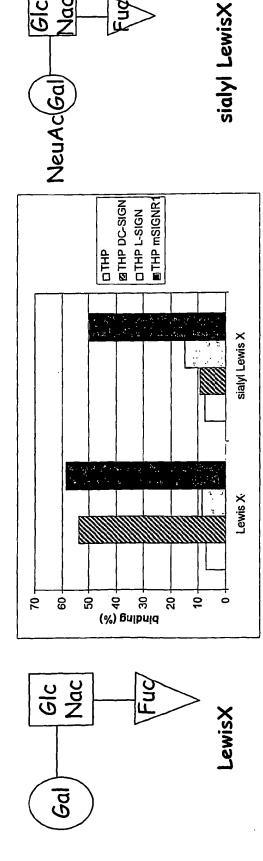


Fig. 33C

## Carbohydrate specificity of DC-SIGN, L-SIGN and mSIGNR1

	DC-SIGN	L-SIGN	mSIGNR1
LewisX	+	$\bigcirc$	+
sialyl LewisX	-	-	+
sulfo LewisX	+	$\bigcirc$	+
LewisY	+	+	+
LewisA	+	+	+
sialyl LewisA	-	-	( <del>+</del> )
sulfo LewisA	+	+	+
LewisB	+	+	+

Fig. 34

### pathogens with mannose-containing carbohydrates bind mSIGNR1

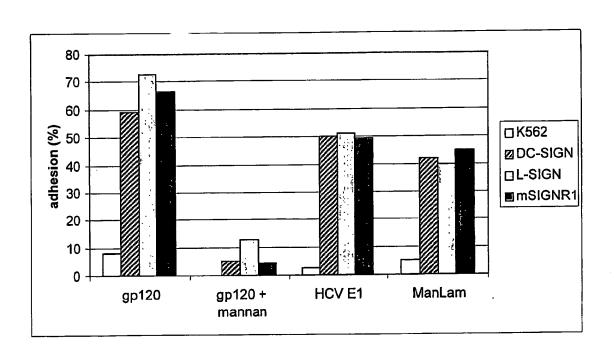


Fig. 35

## DC-SIGN-Fc binds HCV-envelope proteins

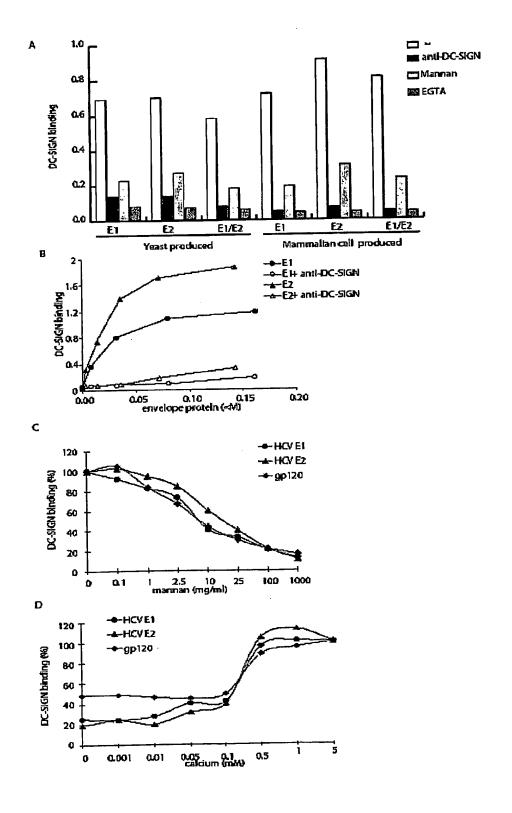
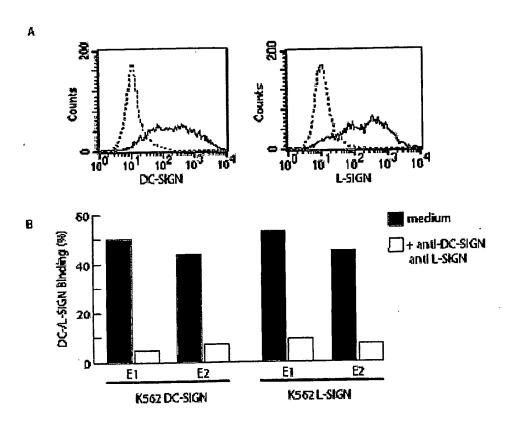


Fig. 36

## Cellular DC-SIGN and L-SIGN bind HCV E1 and E2 proteins

DC-SIGN has similar binding site for gp120 and HCV



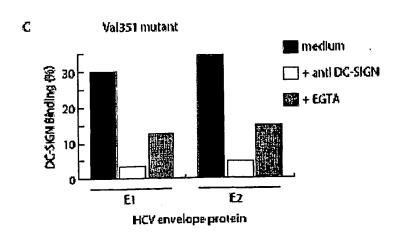


Fig. 36D

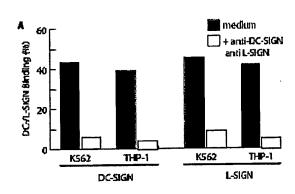
## Essential amino acid residues in DC-SIGN binding to its ligands gp120 and HCV envelope proteins

DC-SIGN mutant				
DC-SIGIV HIULAIIL	gp120	HCV E1	HCV E2	HCV E1/E2
Wild type	% 45	% 50	% 45	% 50
E347Q N349D N365D	4 0 0	3 5 Lig 5 bir	gand 7 nding 9	7 7 9
D366A	3		a <sup>2+</sup> te 2	6
D320A E324A N350A D355A	7 0 1 2	4 4 Si 4 Si	te 1 8	5 6 5 7

Stable K562 transfectamts

Fig. 37

# HCV is internalized by DC-SIGN and L-SIGN; internalization pathway depends on cell-line



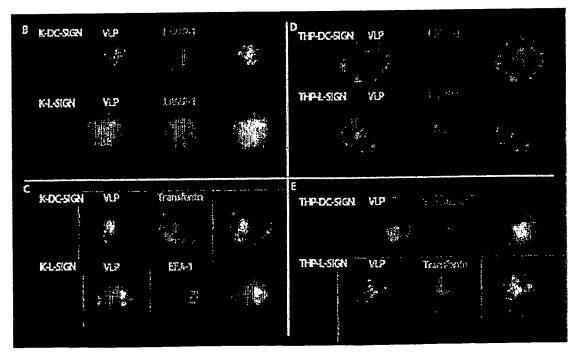


Fig. 38

## Immature and mature DC bind HCV via DC-SIGN

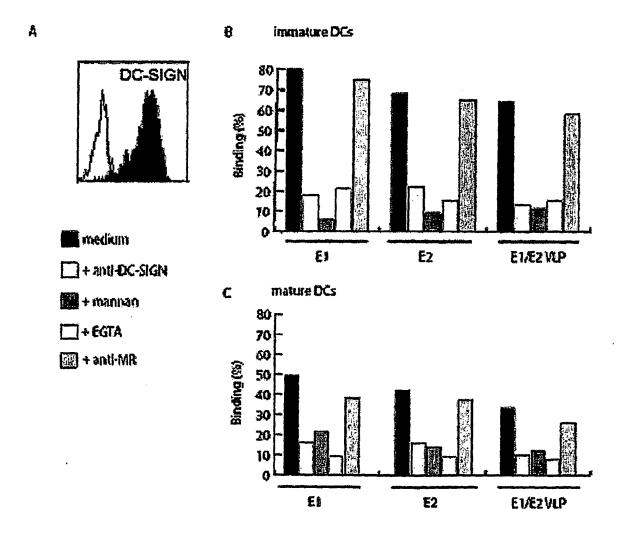


Fig. 39

Immature DC capture and internalize HCV through DC-SIGN; HCV is targeted to the early endosomes

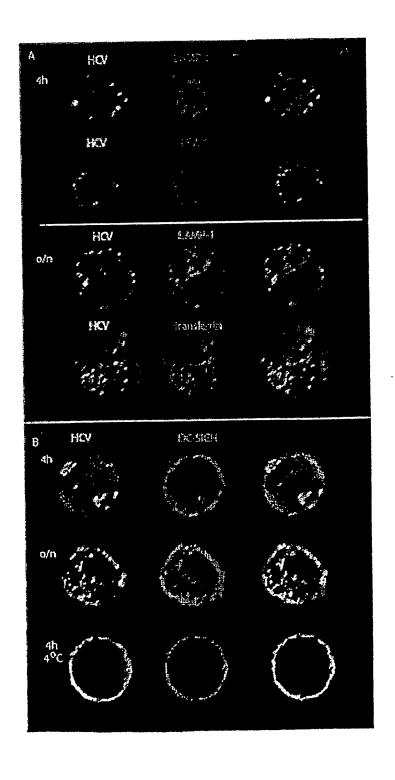


Fig. 40

# Immature DC internalize carbohydrates via DC-SIGN

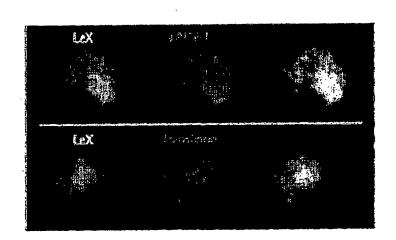


Fig. 41

## HCV binding to Liver sections

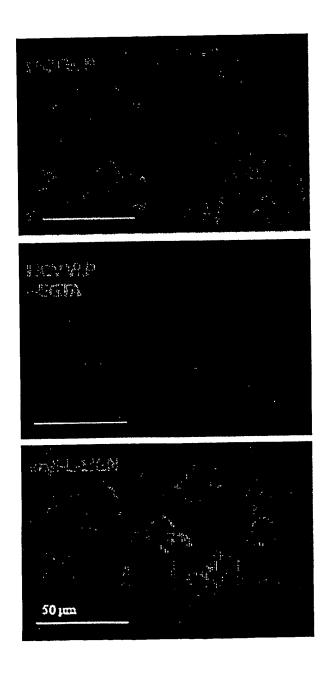


Fig. 42

# Lewis blood group antigens and some of their substructures bind to DC-SIGN.

		<b>.</b>	OD 430
		b	
Structure	Antigen	Lev Lex	
		Fuca1-4GlcNAc	
Fucα1→ 2Galβ1→3GlcNAc	H type 1	Fuca1-3GlcNAc	
·		Fucα1-2Galβ	
Fucα1→ 2Galβ1→4GlcNAc	H type 2	Fuc Gaip1-3GicNAc	_
		Galp1-4GICNAC	
→3 (Galβ1→4GlcNAcβ1→) <sub>n</sub>	<b>i-anti</b> gen	Gal 🚾	
		GicNAc ]	
Galβ1→ 4GlcNAc	Lewis x (Le <sup>x</sup> )	H type 1	
3 <b>↑</b>		,	
Fuca1			OD 494
		c 1	2
Fucα1 → 2Galβ1→ 4GlcNAc	Lewis y (Le <sup>y</sup> )	(Lex) <sub>2</sub>	
3 ↑		(Lex),	
Fuca1			

Fig. 43

# Binding of *H. pylori* is dependent on Lewis antigen expression.

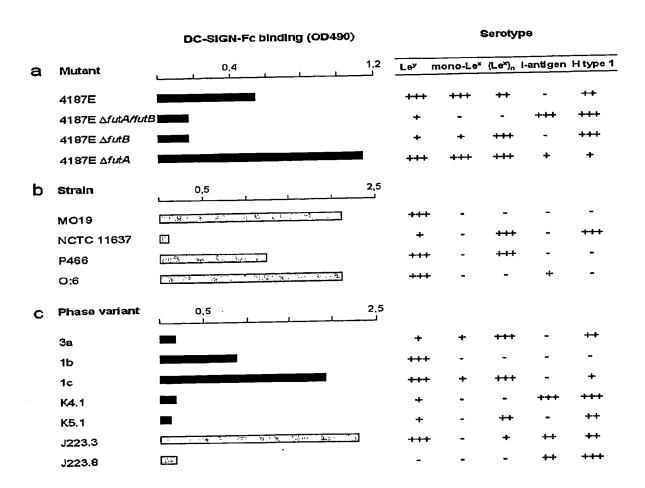


Fig. 44

## LPS phase variation in *H. pylori* occurs in vivo.

а	Strain	Number of colonies (%)	Serotype							
		•	Ley	mono-Lex	(Lex) <sup>u</sup>	i-antigen	H type 1			
	J223.3	6/30 (20)	+++	-	++	++	+++			
	J223.8	24/30 (80)	-	-	•	+++	+++			

b	Strain	Length of C-tract in gene			Serotype				
		futA	futB	Ley	mono-Le <sup>x</sup>	(Le <sub>x</sub> ) <sub>n</sub>	i-antigen	H type 1	
	J223.3	n=9 ("off")	n=10 ("on")	+++	•	++	++	+++	
	J223.8	n=9 ("off")	n=9 ("off")	-	-	-	+++	+++	
	J223.3 ∆ <i>futB</i>	N.D.	N.D	-	-	-	+++	+++	

C	J223.3 futA			J223.3			futB	
	1 61	GAAAGCGCTT	CCCTATTAGA CGCCTTCATA CCATTGAAAA AATGGCCTCT CCCCCTAA (STOP)		1 61	GAAAGCGCTT	CCCTATTAGA CCATTGAAAA CCCCCCTAAA	AATGGCCTCT
	J223	3.8			J223	3.8		
	1 61	GAAAGCGCTT	CCCTATTAGA CGCCTTCATA CCATTGAAAA AATGGCCTCT CCCCCTAA (STOP)	ļ !	1 61	GAAAGCGCTT	CCCTATTAGA CCATTGAAAA CCCCCTAA (	AATGGCCTCT

Fig. 45

# DC-SIGN is expressed on gastric DCs and is the major receptor for Le positive *H. pylori*.

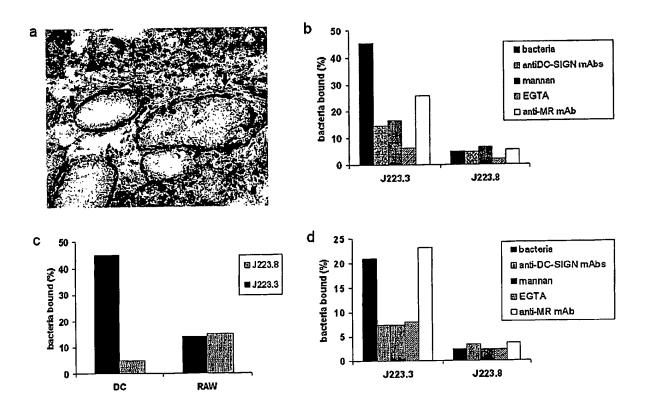
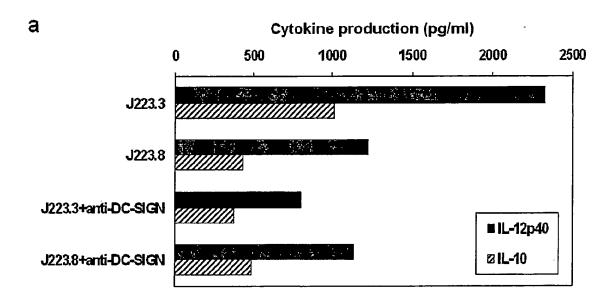


Fig. 46

# Binding of *H. pylori* to induces DC-SIGN-dependent increase of IL-10 and IL-12 production, but no changes in IL12p70.



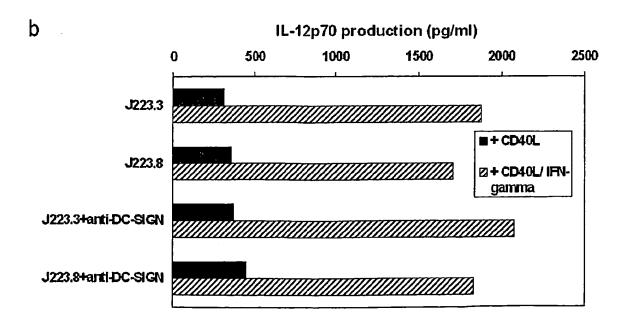
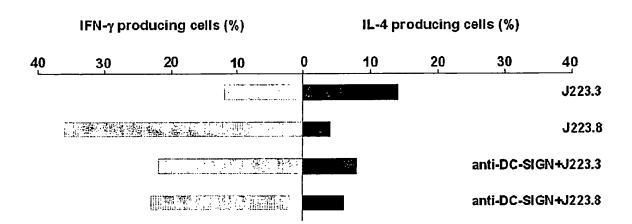


Fig. 47

## Binding of *H. pylori* to DC-SIGN induces skewing of naïve T cells to Th2.



Lactobacilli induce partial DC maturation

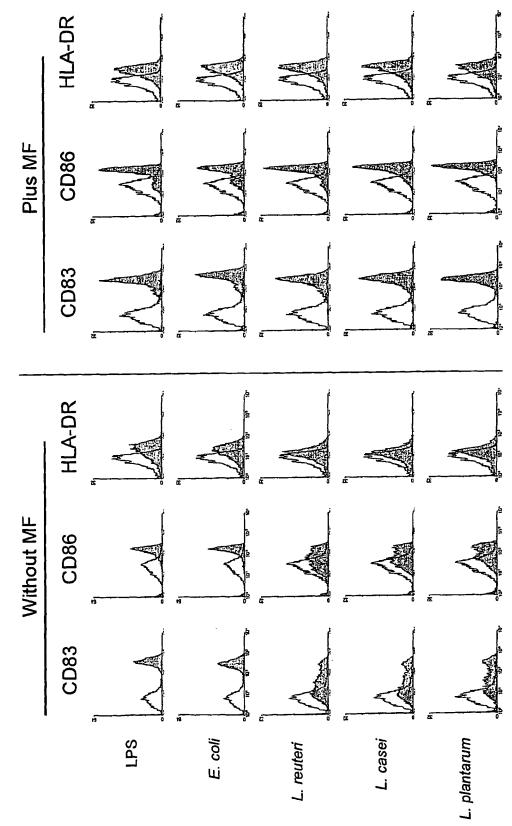


Fig. 48A

Cytokine profiles induced on DC by lactoballi

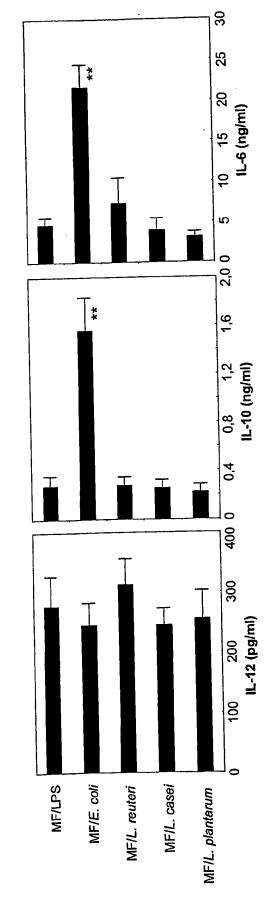


Fig. 48B

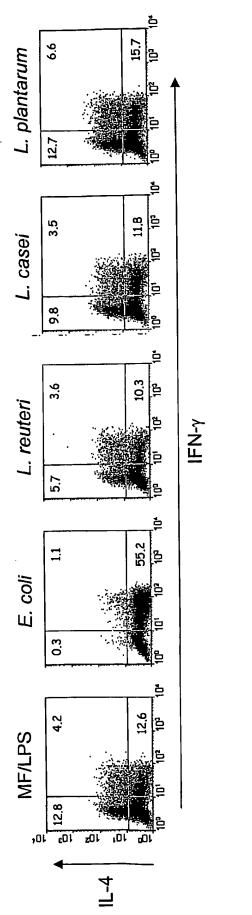


Fig. 49A

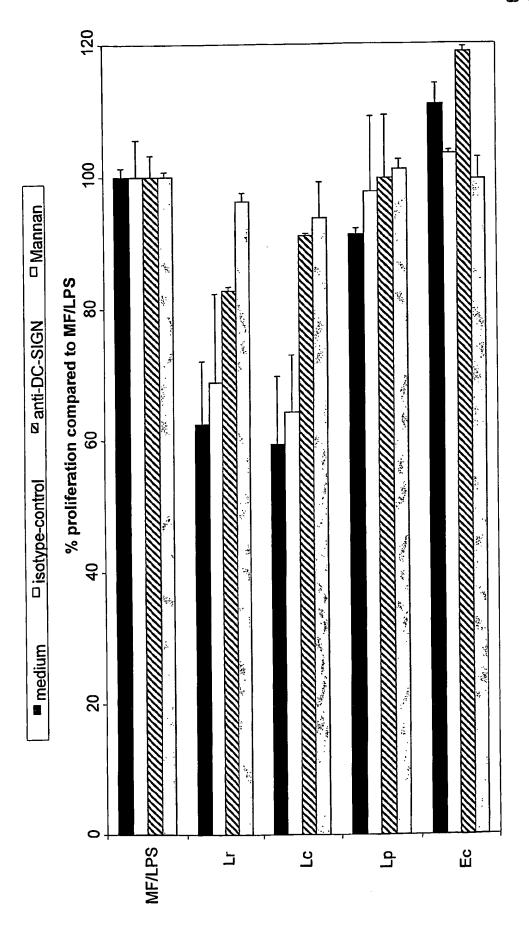
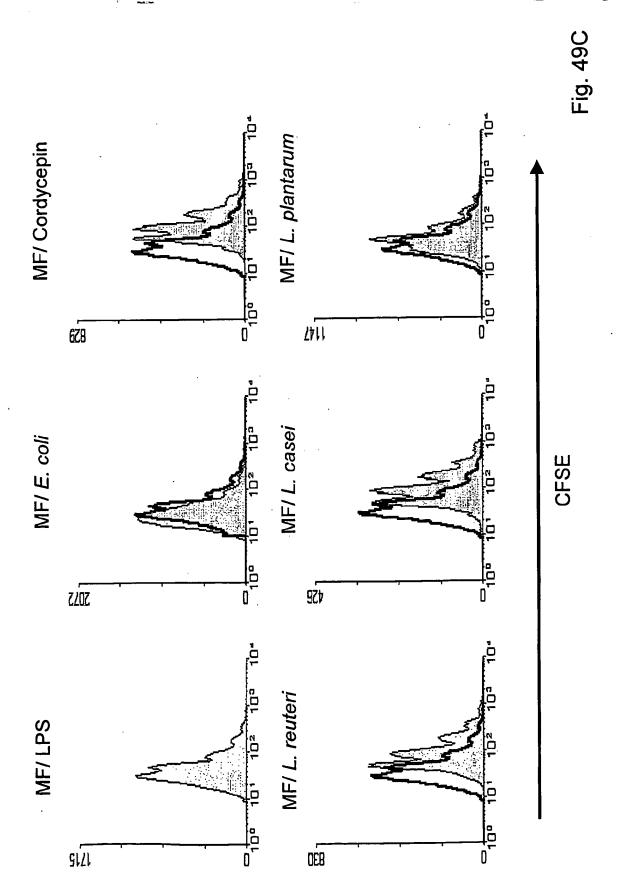


Fig. 49B



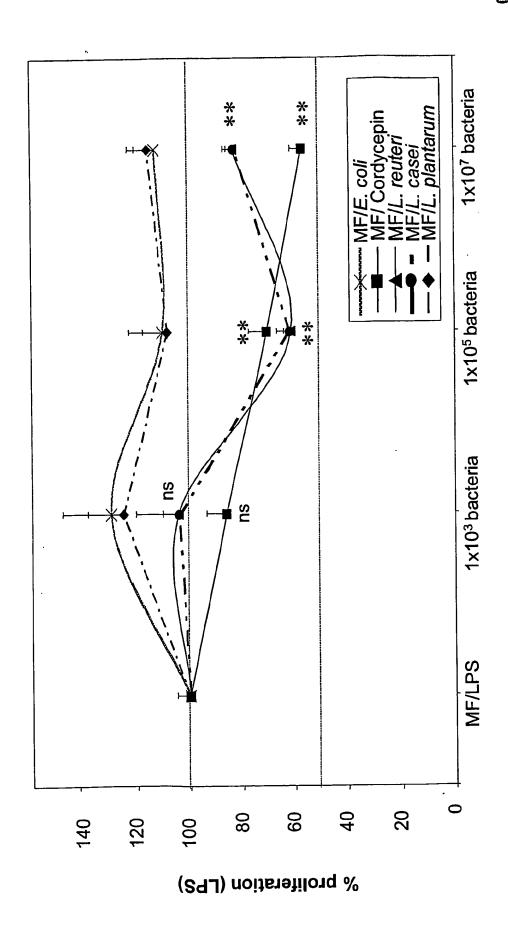
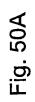
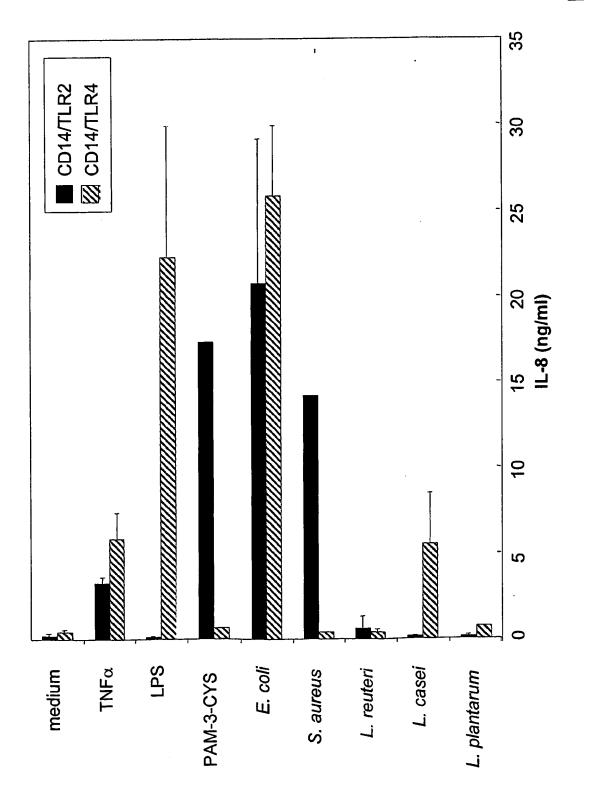
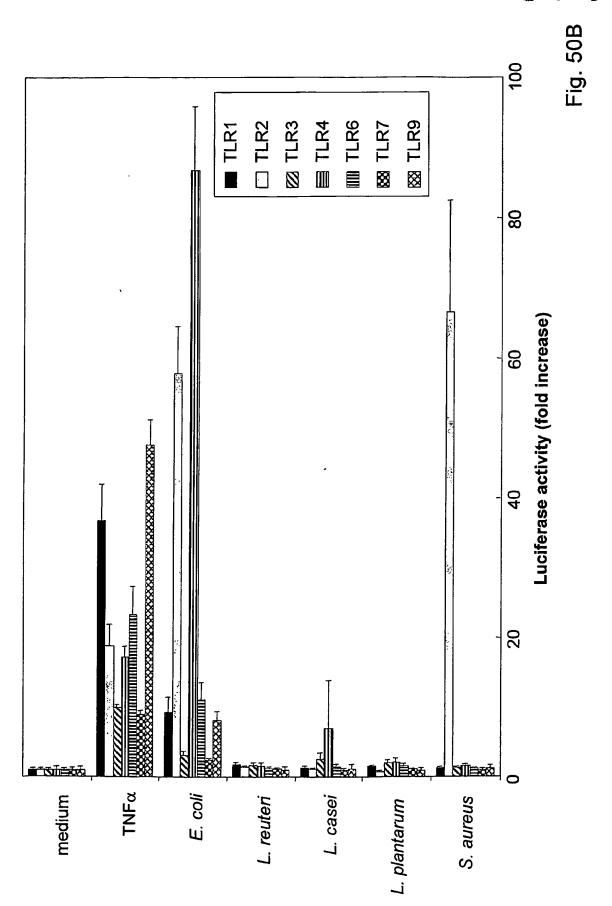
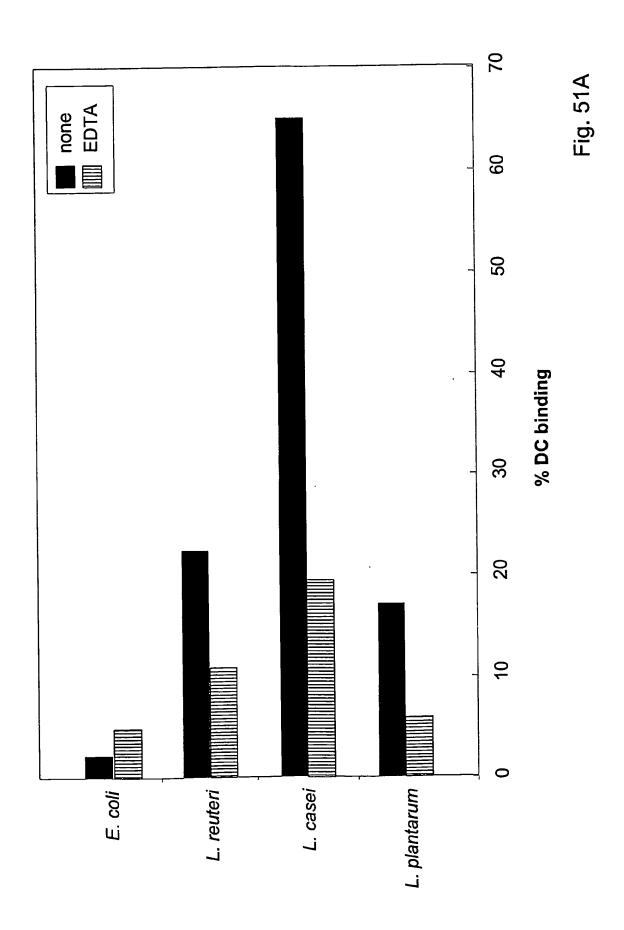


Fig. 49D

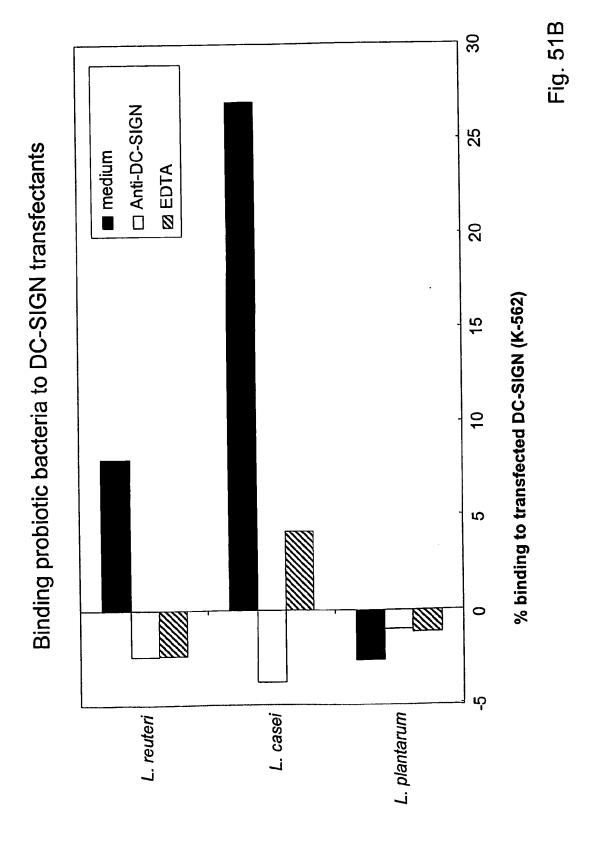




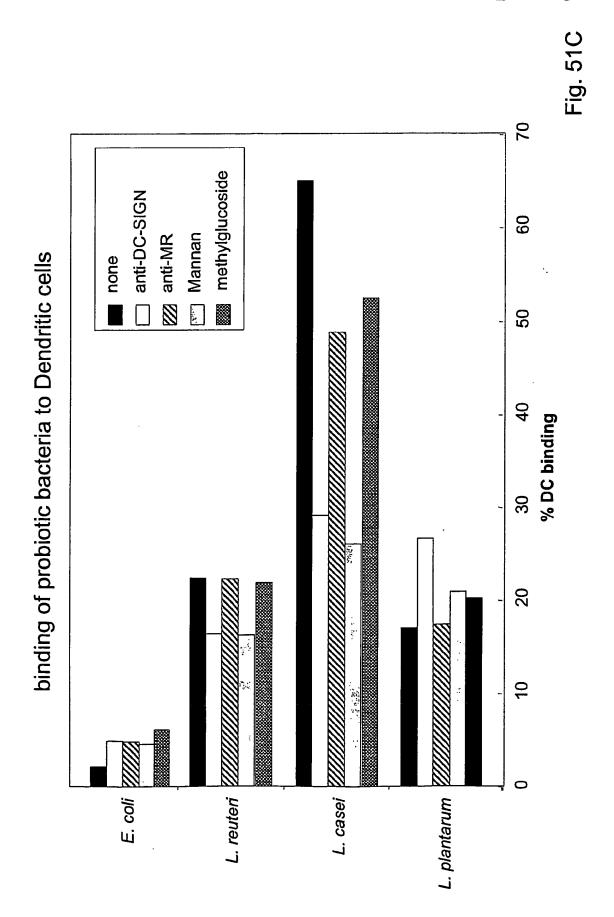




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Induction of T reg cells through targeting DC-SIGN by probiotic bacteria

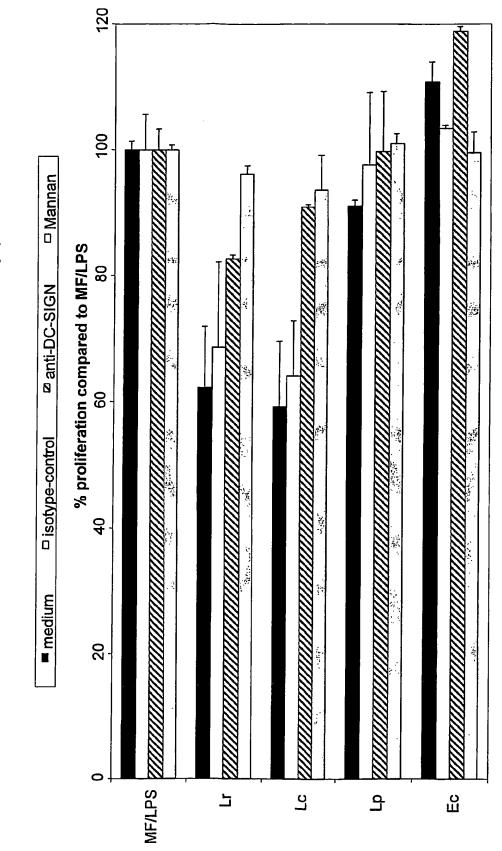


Fig. 52

Fig. 53

# PMN express the DC-SIGN ligand Lewis<sup>x</sup> and bind with high affinity to recombinant DC-SIGN.

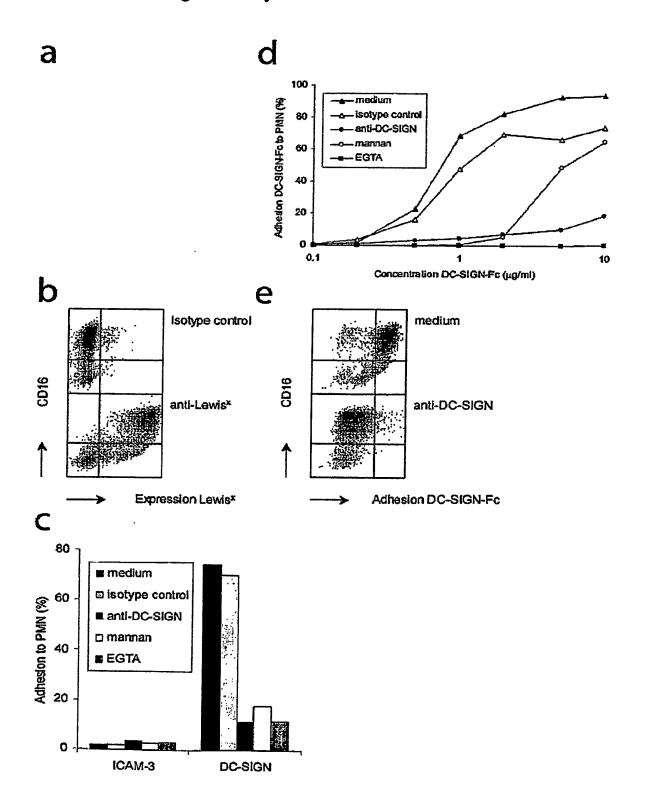


Fig. 54
CEACAM1 expressed on PMN is a ligand of DC-SIGN and binds through its Lewis<sup>x</sup> moieties.

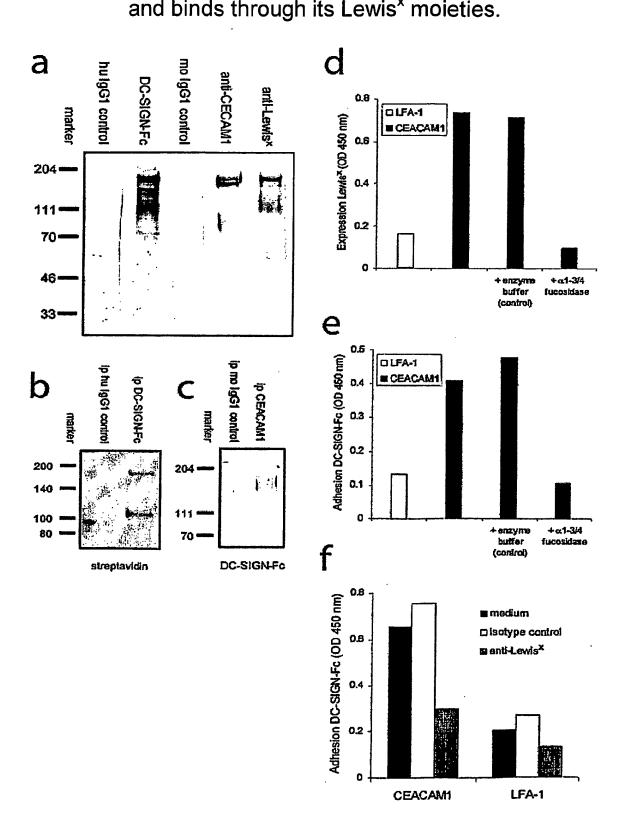
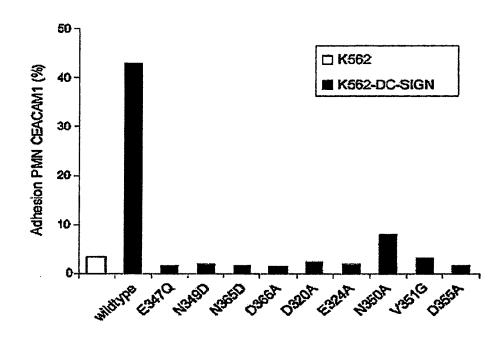


Fig. 55

Cellular DC-SIGN expressed on K562 transfectants and immature DC binds native CEACAM1 from PMN.



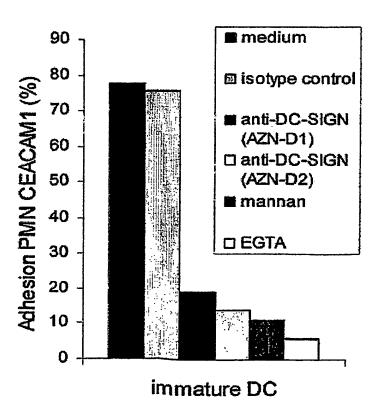
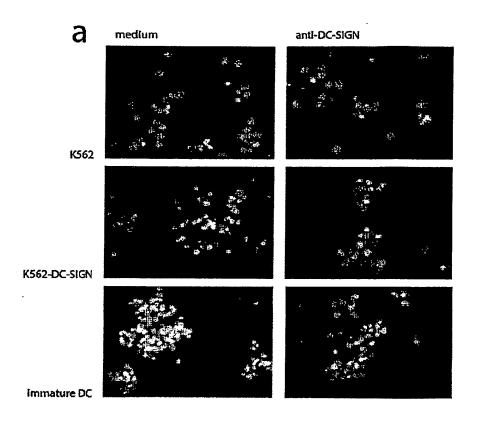


Fig. 56

## DC-SIGN is involved in clustering of DC and PMN.



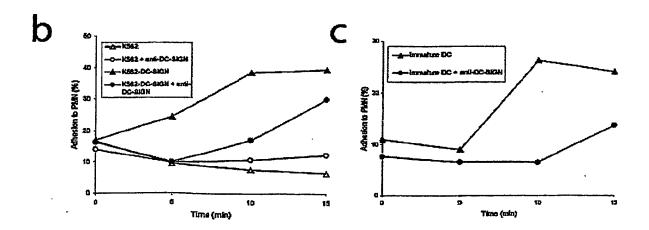


Fig. 57

# Localization of PMN and DC in colonic mucosa of patients with Crohn's disease.

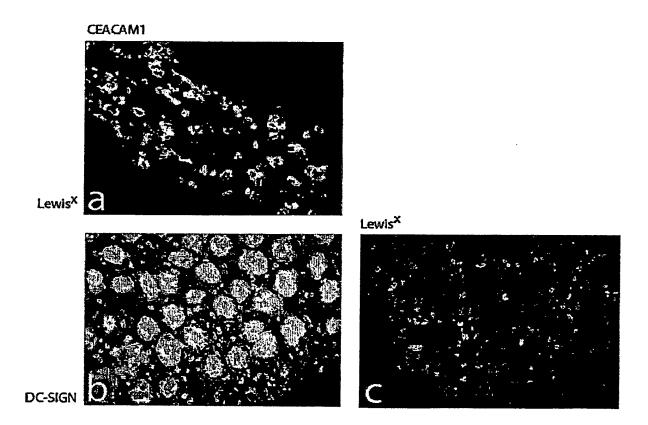
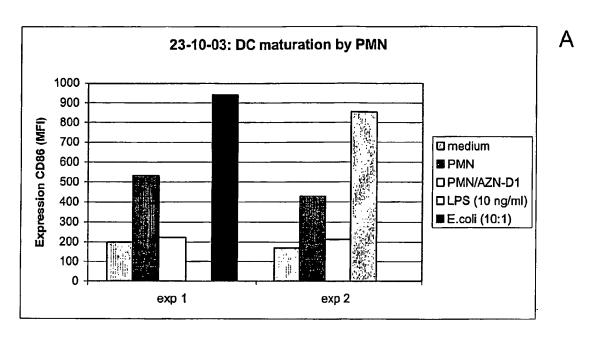
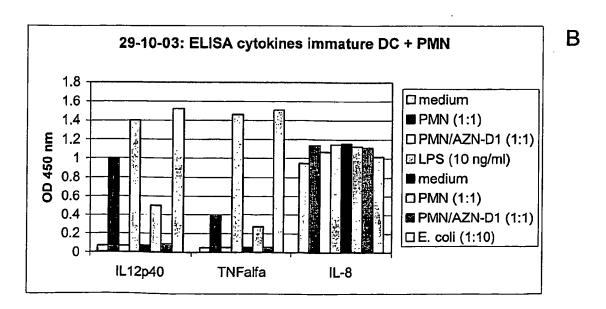


Fig. 58

#### PMN activate immature DC through binding DC-SIGN.



PMN induce upreguation of co-stimulatory CD86 on DC, which is dependent on DC-SIGN binding



PMN induce secretion of inflammatory cytokines by DC, which is dependent on DC-SIGN binding

Fig. 59

DC-SIGN binds Lex expressing CD11b present on neutroph

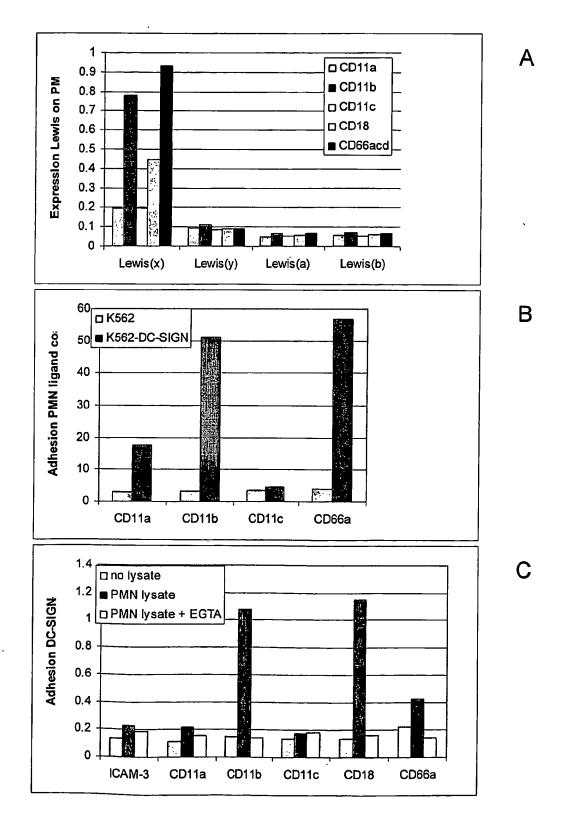
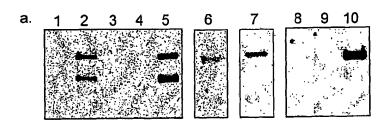


Fig. 59D

#### DC-SIGN bindsCD66acd and CD11b on PMN



- . Biotinylated PMN:
- 1. IP ICAM-3-Fc, IB streptavidin,
- 2. IP DC-SIGN-Fc, IB streptavidin,
- 3. IP anti-DC-SIGN, IB streptavidin,
- 4. IP anti-CD66acd, IB streptavidin,
- 5. IP anti-CD11b, IB streptavidin,
- 6. IP DC-SIGN-Fc, IB anti-CD66acd,
- 7. IP DC-SIGN-Fc, IB anti-CD11b,
- 8 IP anti-DC-SIGN, IB DC-SIGN-Fc,
- 9. IP anti-CD66acd, IB DC-SIGN-Fc,
- 10. IP anti-CD11b, IB DC-SIGN-Fc.
- IB, immunoblotting (detection/binding)
- IP, immunoprecipitation (capture)

Fig. 60

## DC-SIGN binds Lewis on CD11b



#### Biotinylated PMN, IP anti-CD11b, IB streptavidin:

- 1. control
- 2. PNGaseF

#### Biotinylated SW948, IP anti-CD11b, IB DC-SIGN-Fc

- 3. control
- 4. PNGaseF

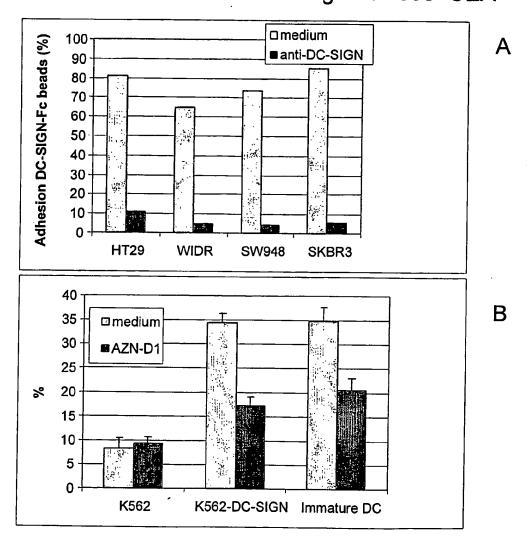
#### Biotinylated PMN, IP anti-CD11b, IB streptavidin:

- 5. control
- 6.  $\alpha$ -1,3/4-Fucosidase

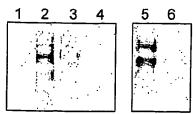
#### Biotinylated PMN, IP anti-CD11b, IB DC-SIGN-Fc

- 7. control
- 8.  $\alpha$ -1,3/4-Fucosidase
- IB, immunoblotting (detection/binding)
- IP, immunoprecipitation (capture)

Fig. 61
DC-SIGN binds tumor cells expressing CD66e
DC-SIGN binds the tumor antigen CD66e=CEA



<u>1 2 3 4</u>

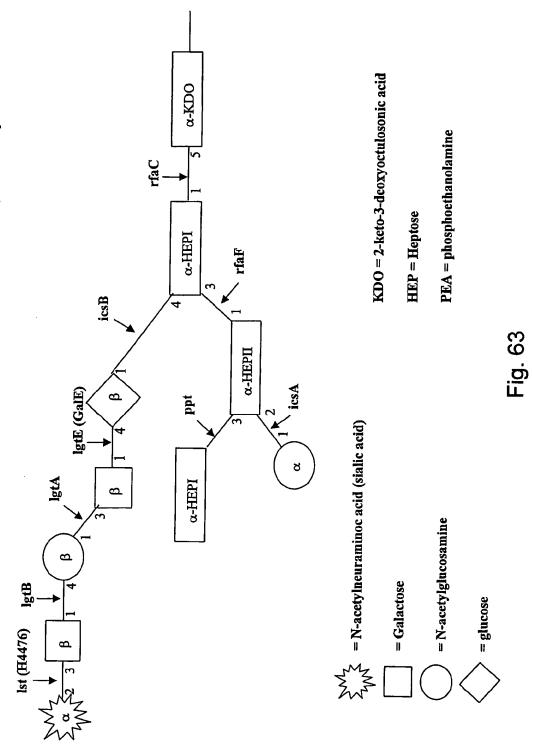


- b. Biotinylated SW948:
- 1. IP ICAM-3-Fc, IB streptavidin,
- 2. IP DC-SIGN-Fc, IB streptavidin
- 3. IP anti-CD66ae, IB streptavidin
- 4. IP anti-CD11b, IB streptavidin
- 5. IP anti-CD66ae, IB DC-SIGN-Fc
- 6. IP anti-CD11b, IB DC-SIGN-Fc
- IB, immunoblotting (detection/binding)
- IP, immunoprecipitation (capture)

Biotinylated SW948, IP anti-Biotinylated SW948, IP anti-CD66a&e, IB streptavidin: CD66a&e, IB DC-SIGN-Fc  $\mathbf{\omega}$ 2. α-1,3/4-Fucosidase 4. α-1,3/4-Fucosidase SW948 lysate СD66 DC-SIGN recognizes Le<sup>x</sup>-Le<sup>y</sup> on CD66e on tumor cells □ no lysate 1. control 3. control CD50 (lgG2b Isotype) СБ66а&е CD11a (lgG1 isotype) 1.2 ö Adhesion DC-SIGN-Fc (OD 450 nm) Fig. 62 Lewis(b) GCD11a (lgG1 isotype) DC-SIGN-Fc ■CD66a&e Lewis(a) Lewis(y) ICAM-3-Fc □ CD66a&e Lewis(x) 0.35 0.25 0.2 Expression Lewis on SW948 (OD 450 nm) Adhesion CD66a&e(OD 450 nm) 4 C

SUBSTITUTE SHEET (RUI F 26)

Neisseria meningitis-GlcNAc specificity



Binding of Neisseria Meningitidis to 293T transfectants (100703)

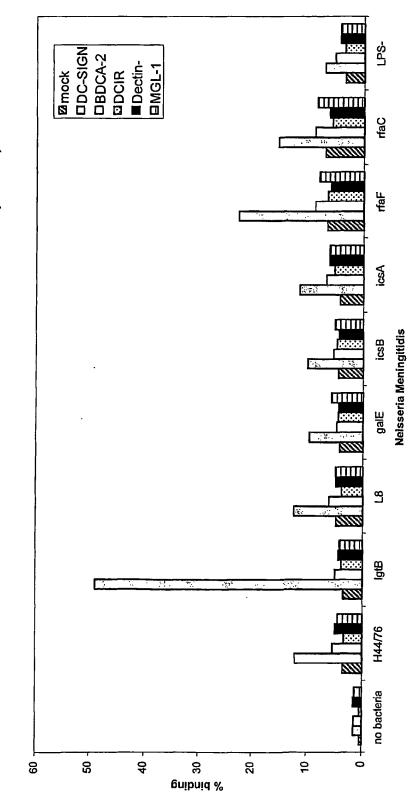


Fig. 64

Binding of Neisseria Meningitidis to DCs (100703)

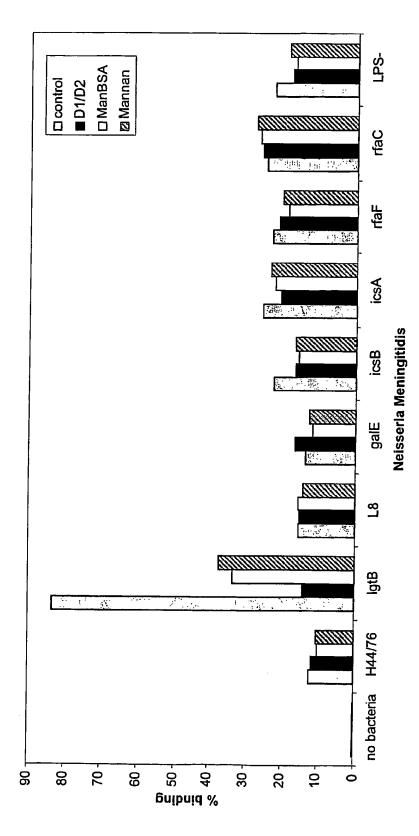
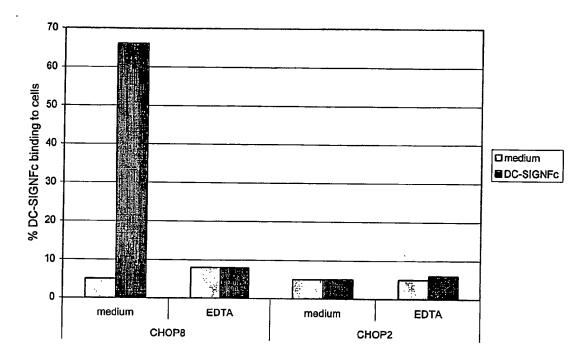


Fig. 65

Fig. 66

#### DC-SIGNFc binds GlcNAc expressing CHOP8 cells



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